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ANAPHYLATOXIN AND ANAPHYLAXIS

XII. STUDIES ON THE CHEMISTRY OF THE BLOOD

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Since the phenomenon of anaphylaxis was first observed many attempts have been made to explain it. Most of these attempts have been purely theoretical, based on biologic data and when any chemical investigation has been made it has involved the antigen primarily, giving the chemistry of the animal tissues and body fluids secondary importance. Conclusions have been reached, therefore, explaining the mechanism of anaphylaxis from observations made on the nature of the antigen and its biochemical possibilities when injected, assuming that the source of the poison (if such) was the antigen.

A more recent view [1, 2] is in favor of considering the constituents of the serum and tissues of the animal body as the source or matrix of the poison or toxic body responsible for the anaphylactic phenomena; and that the toxic body (anaphylatoxin) produced in vitro is identical with that produced in the sensitized animal body by the injection of a suitable antigen. It should be stated, however, that Besredka does not consider anaphylatoxin as having any relation to anaphylactic shock.

In a previous paper [3] it has been shown that amino nitrogen determinations in toxic and control normal serums do not lend support to the theory of proteolysis.

It seemed desirable to follow up this work with a study of the effect, if there is any, produced on the alkaline reserve in the process of poison production and thus to determine whether acidosis has any bearing on anaphylatoxin production or anaphylaxis.

ALKALINE RESERVE AND ANAPHYLATOXIN

In the first series of experiments, anaphylatoxin was prepared with the aid of inulin, following the procedure heretofore employed.[3]

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[1] Bordet and Zunz: Ztschr. f. Immunitätsf., 1914, 23, p. 49.

[2] Novy and DeKruif: "Anaphylatoxin and Anaphylaxis," Jour. Infect. Dis., 1917, 20, p. 499; Jour. Am. Med. Assn., 1917, 68, p. 1524.

[3] De Kruif, P. H., and German, W. M.: Jour. Infect. Dis., 1917, 20, p. 833.

Experiments with Inulin Anaphylatoxin.—Six normal white rats were bled from the heart by means of the usual pipet;[4] the blood was defibrinated, pooled and centrifugated. The supernatant serum was pipetted off and treated as follows:

A. To 5 c c of the serum, 1 c c of 5% inulin suspension (in 0.85% salt solution) was added and the well shaken mixture was then incubated at 38 C. for 15 minutes. It was then centrifugated at 3000 r.p.m. for 15 minutes and the supernatant serum was pipetted off. This was then tested: (1) for toxicity, 1 c c being injected intravenously into a guinea-pig of about 200 gm.; (2) 1 c c was subjected to the Van Slyke method for the determination of plasma bicarbonate, the gasometric CO_2 apparatus being employed.[5]

B. The other portion of 5 c c serum served for control purposes. After adding 1 c c of 0.85% NaCl solution, corresponding to the amount of inulin suspension used in A, the mixture was incubated and centrifugated the same as portion A and at the same time. The clear supernatant serum was then tested (1) for toxicity, and (2) for CO_2 capacity. For each test 1 c c was used, the same as in portion A.

TABLE 1

CARBON DIOXIDE CAPACITY AND ANAPHYLATOXIN IN RAT SERUM TREATED WITH INULIN

Experiment	Percentage CO_2 in		Difference	Intravenous Injection* of 1 C c of	
	Toxic Serum	Control Serum		Toxic Serum	Control Serum
12	43.3	42.9	0.4	Shock and death in 3 min.........	Nil
13	47.3	46.4	0.9	Severe shock........................	Nil
20	39.9	38.1	1.8	Severe shock........................	Nil
21	43.5	41.5	2.0	Shock and death in 3.5 min........	Nil

* The toxicity tests were made on guinea-pigs of 200-250 gm. in this and all subsequent experiments.

It will be seen from Table 1 that the CO_2 capacity of the anaphylatoxic serum is in each instance greater than that of the normal control serum, the average difference being only 1.2%. Clearly, the results show no decrease in the alkaline reserve in the toxic serums as compared with the corresponding control serums.

Experiments with Agar Anaphylatoxin.—A second set of experiments with essentially the same technic throughout but using agar-agar as the anaphylatoxin inducing agent is outlined.

Seven normal white rats were bled from the heart by means of the usual heart pipet, the blood was defibrinated, pooled and centrifugated. The supernatant serum was pipetted off and treated as follows:

A. Seven c c of the serum were treated by the agar sol-gel method. (For this purpose 1.7 c c of the 0.5% agar solution was added to the serum, mixed and iced for 1 hour.) The well shaken mixture was then incubated at 38 C. for 10 minutes. It was then centrifugated at 3,000 r.p.m. for 15 minutes and the supernatant serum was pipetted off. This was then tested: (1) for toxicity, 1 c c being injected intravenously into a guinea-pig of about 200 gm.; and

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[4] Novy and De Kruif: Ibid., 1917, 20, p. 502.

[5] Jour. Biol. Chem., 1917, 30, p. 347.

(2) for CO_2 capacity, 6 c c being subjected to the Van Slyke bicarbonate determination, 1 c c samples being used for each determination.

B. The other portion of 7 c c serum served for control purposes. After adding 1.7 c c of 0.85% salt solution, corresponding to the amount of agar solution used in A, the well shaken mixture was iced for 1 hour and then incubated at 38 C. for 10 minutes. It was then centrifugated at 3,000 r.p.m. for 15 minutes, the same as portion A and at the same time. The clear supernatant serum was then tested for (1) toxicity and (2) for CO_2 capacity. For each test 1 c c was used, the same as in portion A. The results of 4 experiments as outlined are given in table 2.

TABLE 2

CARBON DIOXIDE CAPACITY AND ANAPHYLATOXIN IN RAT SERUM TREATED WITH AGAR

Experiment	Percentage CO_2 in		Difference	Intravenous Injection of 1 C c of	
	Toxic Serum	Control Serum		Toxic Serum	Control Serum
16	43.6	41.6	2.0	Typical death	Nil
3a	26.9	27.9	—1.0	Severe shock	Nil
17	43.3	42.4	0.9	Typical death	Nil
2a	37.1	30.4	6.7	Typical death	Nil

Experiments with Distilled Water Anaphylatoxin.—In a third set of experiments distilled water was used as the anaphylatoxin inducing agent by the method described by Novy and De Kruif.[6]

Three normal white rats were bled from the heart by means of the usual pipet; the blood was defibrinated, pooled and centrifugated. The supernatant serum was pipetted off and treated thus:

A. To 2 c c of serum were added 12 c c of distilled water. After thorough shaking this mixture was incubated at 38 C. for 30 minutes. This was then tested for (1) toxicity, 7 c c or the equivalent of 1 c c of serum being injected intravenously into a guinea-pig of about 200 gm.; and (2) for CO_2 capacity, the Van Slyke plasma bicarbonate method being used as before.

B. For purposes of control, to 2 c c of serum were added 12 c c distilled water, and, after being thoroughly shaken, the mixture was iced for 30 minutes at the same time that A was being incubated. This was then tested (1) for toxicity, 7 c c (or the equivalent of 1 c c of serum) being injected intravenously into a guinea-pig of about 200 gm.; and (2) for CO_2 capacity, the Van Slyke plasma bicarbonate method being used as before.

C. As a further control 5 c c distilled water were incubated as in the preceding and portions of 1 c c were subjected to the Van Slyke plasma bicarbonate method. The result was subtracted from the results obtained in A and B to correct for the dilution resulting from the addition of distilled water and to put the results on the basis of incubated serum.

In both of the experiments given in table 3, the carbon dioxide of the anaphylatoxic serum is slightly greater than that of the control, the average difference being 1.75%, which is practically the same as noted in table 1.

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[6] Jour. Infect. Dis., 1917, 20, p. 807.

TABLE 3

CARBON DIOXIDE CAPACITY AND ANAPHYLATOXIN IN RAT SERUM TREATED WITH DISTILLED WATER

Experiment	Percentage CO_2 in		Difference	Intravenous Injection of 1 C c of	
	Toxic Serum	Control Serum		Toxic Serum	Control Serum
23	22.0	19.5	2.5	Typical shock and death............	Nil
25	11.0	10.0	1.0	Typical shock and death............	Nil

Experiments with Inulin Anaphylatoxin in Guinea-Pig Serum.—A fourth set of experiments, observing the same procedure and technic, but using guinea-pig serum instead of rat serum, yielded results which are given in Table 4.

For each experiment a guinea-pig was bled from the heart by means of a blood pipet; the blood was defibrinated, centrifugated and the supernatant serum was pipetted off. It was then treated as follows:

A. To 5 c c of the serum 1 c c of 5% inulin suspension in 0.85% salt solution was added and the well shaken mixture was then incubated at 38 C. for 20 minutes. It was then centrifugated at 3,000 r.p.m. for 15 minutes and the supernatant serum was pipetted off. This was then tested (1) for toxicity, 2.5 c c being injected intravenously into a guinea-pig of about 200 gm.; and (2) for CO_2 capacity, being subjected to the Van Slyke plasma bicarbonate method as before.

B. For purposes of control, to another portion of 5 c c of the serum was added 1 c c of 0.85% salt solution. After thorough shaking the mixture was incubated at 38 C. for 20 minutes at the same time as A; was centrifugated at 3,000 r.p.m. for 15 minutes and the clear supernatant serum pipetted off. This was then tested (1) for toxicity, 2.5 c c being injected intravenously into a guinea-pig of about 200 gm.; and (2) for CO_2 capacity, the Van Slyke plasma bicarbonate method being used as before.

TABLE 4

CARBON DIOXIDE CAPACITY AND ANAPHYLATOXIN IN GUINEA-PIG SERUM TREATED WITH INULIN, AGAR AND DISTILLED WATER

	Experiment	Percentage CO_2 in		Difference	Intravenous Injection of 1 C c of	
		Toxic Serum	Control Serum		Toxic Serum	Control Serum
Inulin............	14b	31.2	32.1	0.9	Nil........................	Nil
Inulin............	15	36.6	35.7	0.9	Severe shock..............	Nil
Agar..............	14a	44.4	41.7	2.7	Typical shock and death.	Nil
Distilled water....	22	37.1	30.4	6.7	Nil........................	Nil

The fact that guinea-pig serum is less easily made toxic explains the poor grade of anaphylatoxin produced. The average difference between the normal control and anaphylatoxic serum in CO_2 capacity is here only 0.9%, which is small enough to be within the limits of experimental error.

Experiments with Rat Plasma Inulin Anaphylatoxin.—For each experiment 5 or 6 normal white rats were bled from the heart, the blood being received directly into a centrifuge tube containing a few milligrams of powdered potassium oxalate. After being centrifugated, the supernatant plasma was pipetted off and pooled. This was divided into two equal portions:

A. To 3 c c of plasma was added 0.5 c c of 5% inulin suspension (in 0.85% salt solution). The well shaken mixture was incubated at 38 C. for 15 minutes. It was then centrifugated at 3,000 r.p.m. for 15 minutes and the supernatant plasma was pipetted off. This was then tested (1) for toxicity, 1 c c being injected intravenously into a guinea-pig of about 200 gm.; and (2) for CO_2 capacity, being subjected to the Van Slyke plasma bicarbonate method in 1 c c portions.

B. For control purposes, to 3 c c of the plasma was added 0.5 c c of 0.85% salt solution and the mixture was incubated at 38 C. for 15 minutes at the same time as A. After being centrifugated for 15 minutes at 3,000 r.p.m. the supernatant plasma was pipetted off and tested (1) for toxicity, 1 c c being injected intravenously into a guinea-pig of about 200 gm.; and (2) for CO_2 capacity, being subjected to the Van Slyke plasma bicarbonate method as usual. The results of 5 such experiments are given in table 5, all carried out under exactly the same conditions throughout.

TABLE 5

CARBON DIOXIDE CAPACITY AND ANAPHYLATOXIN IN RAT PLASMA TREATED WITH INULIN

Experiment	Percentage CO_2 in		Difference	Intravenous Injection of 1 C c of	
	Toxic Serum	Control Serum		Toxic Serum	Control Serum
1	23.9	24.2	0.3	Typical shock and death............	Nil
2	29.3	28.5	0.8	Typical shock and death............	Nil
3	24.7	25.6	0.9	Severe shock.........................	Nil
4	29.4	27.5	1.9	Typical shock and death............	Nil
5	39.8	37.9	0.9	Severe shock.........................	Nil

Here again it can be seen that the average difference in the CO_2 capacity between the anaphylatoxic and normal control plasma is 0.9%, a figure that is sufficiently small to be within the range of experimental error. In every case the plasma was made toxic by incubation while no change in the plasma alkaline reserve kept pace with it.

Experiments with Rat Plasma-Distilled Water Anaphylatoxin.—Finally, rat plasma was made toxic by the use of distilled water, the procedure being the same as that employed with rat serum (Table 3).

For each experiment 5 or more normal rats were bled from the heart, the blood being oxalated by the addition of powdered potassium oxalate, as given in the foregoing, and centrifugated. The supernatant plasma was pipetted off and pooled. This was divided into two portions.

A. To 2 c c plasma were added 10 c c of distilled water. This mixture was shaken for one minute and incubated for 20 minutes at 38 C. It was then tested (1) for toxicity, 6 c c (or the equivalent of 1 c c of plasma) being injected intravenously into a guinea-pig of about 200 gm., and (2) for CO_2 capacity, being subjected in 2 c c samples to the Van Slyke plasma bicarbonate method.

B. For purposes of control, to 2 c c of plasma were added 10 c c distilled water and after being shaken thoroughly the mixture was iced for 30 minutes at the same time that A was being incubated. This was then tested (1) for toxicity, 6 c c (or the equivalent of 1 c c of serum) being injected intravenously into a guinea-pig of about 200 gm.; and (2) for CO_2 capacity, being subjected to the Van Slyke plasma bicarbonate method in 2 c c samples.

C. As a further control, 5 c c of distilled water were subjected to the Van Slyke plasma bicarbonate method. The result was deducted from results obtained in A and B to correct for the addition of distilled water and to put the results on the basis of undiluted plasma.

The results of 6 such experiments, all carried out under exactly the same conditions, are given in Table 6.

TABLE 6

CARBON DIOXIDE CAPACITY AND ANAPHYLATOXIN IN RAT PLASMA TREATED WITH DISTILLED WATER

Experiment	Percentage CO_2 in		Difference	Intravenous Injection of 1 C c of	
	Toxic Serum	Control Serum		Toxic Serum	Control Serum
6	35.9	38.7	2.8	Typical shock and death	Nil
7	24.4	57.0	32.6	Typical shock and death	Nil
8	53.7	53.7	0.0	Severe shock	Nil
9	47.3	47.3	0.0	Severe shock	Nil
10	48.1	50.0	1.9	Severe shock	Nil
11	66.2	55.7	10.5	Typical shock and death	Nil

In this series of experiments there is an average difference of 7.8% in the CO_2 capacity between the anaphylatoxic and control plasma. Such a discrepancy can be accounted for by the fact that CO_2 determinations on 2 c c of the experimental unknown which represents only 0.2 c c of plasma are rendered more liable to error by the factors, dilution and extremely small quantities. The experimental error is therefore large.

Briefly, the results of comparison of CO_2 capacity of anaphylatoxic and normal control serum and plasma are summed up as follows:

Of 25 experiments, in 16 the percentage of bound CO_2 was greater in the anaphylatoxic serum as compared with the normal control; and was less in 7 instances, and in 2, was equal to the controls.

The small differences in percentage of CO_2 which were obtained can easily be within the limits of experimental error.

These facts point to the exclusion of the alkaline reserve as a factor in the mechanism of anaphylatoxin production in serum or plasma.

BLOOD AMMONIA AND ANAPHYLATOXIN

Since, in acidosis there is an increase in blood ammonia as a compensatory attempt to protect the alkaline reserve, a series of blood ammonia determinations were carried out as a check on the foregoing determinations of CO_2 capacity of serum and plasma.

Experiments with Rat Serum-Agar Anaphylatoxin, Inulin Anaphylatoxin, and Distilled Water Anaphylatoxin.—Six normal white rats were bled from the heart by means of a heart pipet, the blood was defibrinated and pooled. This was divided into two portions, A and B.

A. Four and one-half c c serum were treated with agar by the sol-gel method (1 c c of 0.5% agar solution being added, the whole being shaken for 1 minute, then iced for 1 hour and incubated for 10 minutes at 38 C. It was then centrifugated at 3,000 r.p.m. for 15 minutes and the supernatant serum pipetted off. This was then tested (1) for toxicity, 1 c c being injected intravenously into a guinea-pig of about 200 gm., and (2) for ammonia content, being subjected to Folin's method for the determination of blood ammonia.

B. For control purposes 4.5 c c serum were treated with 0.1 c c physiologic salt solution, the mixture shaken for 1 minute, iced for 1 hour at the same time as A, then incubated for 10 minutes at 38 C. It was then centrifugated at 3,000 r.p.m. and the supernatant serum pipetted off. This was then tested (1) for toxicity, 1 c c being injected intravenously into a guinea-pig of about 200 gm., and (2) for ammonia content, being subjected to Folin's method.

Eight such experiments were carried out using agar, inulin and distilled water as inducing agents. The plan and technic in each case were essentially the same. The results of these experiments can be seen and compared in table 7. Separate pooled rat serums were used in each experiment, except in experiment 2a in which guinea-pig serum was used.

TABLE 7

BLOOD AMMONIA AND ANAPHYLATOXIN

	Experiment	Mg. NH_3 per 100 C c Blood in		Difference	Intravenous Injection of 1 C c of	
		Toxic Serum	Control Serum		Toxic Serum	Control Serum
Agar	3a	0.20	0.20	0.0	Typical shock and death	Nil
Agar	22	2.5	2.5	0.0	Typical shock and death	Nil
Agar	2a	0.75	0.75	0.0	Typical shock and death	Nil
Inulin	4a	0.60	1.0	0.4	Typical shock and death	Nil
Inulin	20	1.0	1.0	0.0	Typical shock and death	Nil
Inulin	21	1.0	1.0	0.0	Typical shock and death	Nil
Distilled water	23	1.0	1.5	0.5	Typical shock and death	Nil
Distilled water	25	1.0	1.0	0.0	Typical shock and death	Nil

It will be seen from table 7 that there is practically no change in the ammonia content of toxic serums as compared with corresponding controls and hence the conclusion is justified that the mechanism of anaphylatoxin production in serum does not involve any alteration in the amount of ammonia.

ALKALINE RESERVE AND SPECIFIC ANAPHYLAXIS

Since no alteration can be detected in the alkaline reserve in vitro, it appeared desirable to determine whether any such change took place in the animal body. Here a difference, notably a decrease, in the plasma bicarbonate content during or after acute anaphylactic shock would point to acidosis, if such a condition were concerned with the phenomenon of shock. With this in view, two series of experiments were carried out, the first series in rabbits which had been sensitized against a protein antigen (specific anaphylaxis). The second set was carried out on normal rabbits in which anaphylactic symptoms were induced by the injection of a nonspecific antigen as agar (nonspecific anaphylaxis). Rabbits were selected as experimental animals even though they responded with difficulty to attempts to induce anaphylaxis because the volume of blood required for plasma determinations did not permit the use of the smaller laboratory animals.

Outline of Experiment.—Specific Anaphylactic Shock.—A normal Belgian rabbit received intravenously 10 c c normal rat serum every 4 days for 3 weeks. Seven days were allowed to elapse, at the end of which an excellent precipitin reaction was obtained against normal rat serum. The sensitized rabbit was attached to a Latapie board and with aseptic technic the left carotid artery was exposed. In this a sterile paraffined cannula was introduced and secured by means of a ligature.

A. Ten c c blood were withdrawn through the cannula and received in a centrifuge tube containing 10 mg. powdered potassium oxalate. The oxalated blood was then centrifugated at 3,000 r.p.m. for 15 minutes and the supernatant plasma pipetted off. This was subjected to the Van Slyke bicarbonate determination.

Twenty-five c c normal rat serum were then injected slowly into the ear vein. This was followed in 3½ minutes by a profound anaphylactic shock. When the symptoms of shock had nearly subsided and immediately before death:

B. Ten c c blood were withdrawn through the cannula and received in a centrifuge tube containing 10 mg. powdered potassium oxalate. The oxalated blood was then centrifugated at 3,000 r.p.m. for 15 minutes and the supernatant plasma pipetted off. This was then subjected to the Van Slyke plasma bicarbonate determination. Necropsy showed delayed coagulation (25 minutes); no thrombi were found in the heart or great vessels.

Six such experiments on specific anaphylactic shock were carried out. The sensitizing was done in the same manner in every case. In 3 instances rat serum was used as antigen, in 2 experiments guinea-pig serum was used and in one case guinea-pig whole blood was used. The results of this series are outlined in table 8.

TABLE 8

ALKALINE RESERVE AND SPECIFIC ANAPHYLACTIC SHOCK IN RABBITS

Experiment	Percentage CO_2 in Serum		Sensitized to	Difference	Result
	Before Shock	After Shock			
18	14.2	16.0	Rat serum	1.8	Typical shock and death
18b	22.1	18.3	Rat serum	3.8	Typical shock
19	46.5	33.0	Rat serum	13.5	Typical shock and death
24	35.4	36.1	Guinea-pig serum	0.7	Typical shock
26	48.5	49.8	Guinea-pig serum	1.3	Typical shock and death
27	42.2	41.9	Guinea-pig whole blood	0.3	Typical shock and death

In every case an intense shock was produced. In two instances recovery followed, but this is not unusual in anaphylaxis in rabbits. In this series the average difference in plasma bicarbonate content before and after shock is not only very small but inconstant, frequently being greater after shock than before. Both of these facts are incompatible with the idea that the alkaline reserve is involved.

ALKALINE RESERVE AND NON-SPECIFIC ANAPHYLACTIC SHOCK

Since anaphylactic shock can be induced in normal animals by the injection of suitable substances, a series of experiments were carried out on normal rabbits using agar as the inducing agent. This was prepared according to the method of Novy and De Kruif.[7] The routine of procedure was essentially the same as in the experiments with sensitized animals except that no preliminary sensitization was required.

Outline of Experiment—Nonspecific Anaphylactic Shock.—A normal Belgian rabbit of about 2 kg. weight was attached to a Latapie board. With aseptic technic, 10 c c blood were withdrawn by heart puncture and transferred to a centrifuge tube containing 10 mg. powdered potassium oxalate. The oxalated blood was centrifugated at 3,000 r.p.m. for 15 minutes and the supernatant plasma was pipetted off. This was subjected to the Van Slyke plasma bicarbonate determination.

Twenty-five c c agar sol-gel was injected slowly into the ear vein. This was followed in 3 minutes by a profound anaphylactic shock. When the symptoms had subsided and immediately before death 10 c c blood were withdrawn by heart puncture and transferred to a centrifuge tube containing 10 mg. powdered potassium oxalate. The oxalated blood was then centrifugated at 3,000 r.p.m. for 15 minutes and the supernatant plasma pipetted off. This was then subjected to the Van Slyke plasma bicarbonate method.

Necropsy showed fluid blood, a very small pericardial hemorrhage and no thrombi in the heart or the great vessels.

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[7] Jour. Infect. Dis., 1917, 20, p. 631.

The difference in plasma bicarbonate content before and after shock was in this case 1%. A series of 4 such experiments, identical in detail and technic are given in table 9.

TABLE 9

ALKALINE RESERVE AND NONSPECIFIC ANAPHYLAXIS IN NORMAL RABBITS AFTER AGAR INJECTION

Experiment	Percentage CO_2 in Plasma		Difference	Result of Injection
	Before Injection	After Injection		
28	40.0	40.0	0.0	Typical shock and death
29	32.5	30.8	1.7	Typical shock and death
30	32.5	31.5	1.0	Typical shock and death
31	27.0	28.0	1.0	Typical shock and death

In this series with an average difference in plasma bicarbonate content of 0.9%, before and after shock, and with one instance in the 4 in which the percentage is greater after than before, the obvious conclusion to make is that the alkaline reserve is not involved in the phenomenon.

BLOOD AMMONIA AND SPECIFIC ANAPHYLAXIS

Again as a check on the plasma bicarbonate determinations, in anaphylaxis before and after shock, micro determinations of ammonia[8] were carried out. The technic employed was essentially the same as that used for specific anaphylaxis except that ammonia determinations were made instead of those for carbon dioxide. Four such experiments are outlined in table 10.

TABLE 10

BLOOD AMMONIA AND SPECIFIC ANAPHYLACTIC SHOCK IN RABBITS

Experiment	Mg. NH_3 per 100 C c in Blood		Difference	Sensitized to	Result of Injection
	Before Injection	After Injection			
19	0.5	0.45	0.05	Rat serum...............	Typical shock
24	0.8	0.8	0.00	Guinea-pig serum........	Typical shock
26	0.32	0.30	0.02	Guinea-pig serum........	Typical shock and death
27	0.4	0.5	0.10	Guinea-pig whole blood..	Typical shock and death

The average difference is seen to be 0.64 mg. ammonia per 100 c c blood. This again points to the same conclusion as was reached in anaphylatoxin and ammonia content, namely, that the blood ammonia is not in any way disturbed in the process.

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[8] Folin and Macallum: Jour. Biol. Chem., 1912, 11, p. 523.

SUMMARY AND CONCLUSIONS

In the production of anaphylatoxin in vitro in serum or in plasma there is no demonstrable disturbance in the alkaline reserve as shown by the Van Slyke plasma bicarbonate method.

Likewise micro determinations of blood ammonia showed no variations in this constituent in anaphylatoxic serums as compared with normal serums.

In experimental anaphylaxis, either specific or nonspecific, no disturbance in the alkaline reserve could be demonstrated. Further, in these conditions, no variations in the blood ammonia could be detected. The conclusion that suggests itself is that the alkaline reserve is not involved in any demonstrable manner in the phenomenon of anaphylatoxin production and anaphylaxis.

These results are corroborative of those obtained in the study of amino nitrogen in relation to anaphylatoxin.[3] Therefore, the general conclusion to be drawn is that anaphylatoxin production is not accompanied by changes in amino nitrogen, in blood ammonia, or in the alkaline reserve.